

=> s l11 and mutant

L15 84 L11 AND MUTANT

=> s l15 and ovalbumin

L16 10 L15 AND OVALBUMIN

=> dup remove l16

PROCESSING COMPLETED FOR L16

L17 2 DUP REMOVE L16 (8 DUPLICATES REMOVED)

=> d l17 1-2 cbib abs

L17 ANSWER 1 OF 2 MEDLINE DUPLICATE 1
2001354948 Document Number: 21178697. PubMed ID: 11282993. Evidence for

a role of ganglioside GM1 in antigen presentation: binding enhances presentation of Escherichia coli enterotoxin B subunit (**EtxB**) to CD4(+) T cells. Nashar T O; Betteridge Z E; Mitchell R N. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, UK.) INTERNATIONAL IMMUNOLOGY, (2001 Apr) 13 (4) 541-51. Journal code: AY5; 8916182. ISSN: 0953-8178. Pub. country: England; United Kingdom. Language: English.

AB Successful antigen presentation by antigen-presenting cells is governed by a number of factors including the efficiency of antigen capture by cell-surface receptors, targeting to compartments of antigen processing, surface expression of MHC II-peptide complexes and presence of co-stimulatory signals. Ganglioside GM1 is an important component of membrane glycosphingolipids, and has been implicated in cell differentiation, apoptosis and signal transduction pathways. Using the B subunit of Escherichia coli enterotoxin (**EtxB**), a potent immunogen that binds GM1 with high affinity, and a non-binding mutant of **EtxB**, **EtxB**(G33D), we demonstrate that GM1 is intimately involved in several aspects of antigen presentation. Thus, GM1-mediated presentation of **EtxB** by B cells and CD11c(+) dendritic cells (DC) significantly enhanced the proliferation

and cytokine expression of **EtxB**-specific CD4(+) T cells. Investigation regarding potential mechanisms revealed that **EtxB** binding directly augments the expression of MHC class II on B cells, and fractionation of B cells demonstrated that **EtxB** binding to GM1 results in rapid internalization and targeting to class II-rich compartments. GM1-mediated uptake of antigens and access to class II compartments in B cells can be exploited to significantly enhance the presentation of ovalbumin-conjugated to **EtxB**. These results demonstrate that GM1 can play an important role in antigen presentation via the MHC II pathway.

L17 ANSWER 2 OF 2 MEDLINE DUPLICATE 2
96324796 Document Number: 96324796. PubMed ID: 8671661. Cross-linking of cell surface ganglioside GM1 induces the selective apoptosis of mature CD8+ T lymphocytes. Nahar T O; Williams N A; Hirst T R. (Research School of Biosciences, University of Kent, Canterbury, UK.) INTERNATIONAL IMMUNOLOGY, (1996 May) 8 (5) 731-6. Ref: 24. Journal code: AY5; 8916182. ISSN: 0953-8178. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Gangliosides are glycosphingolipids found ubiquitously on the surface of mammalian cells. They contain a ceramide tail that is inserted into the membrane and exposed carbohydrate and sialic acid moieties. The non-toxic B subunit oligomer (**EtxB**) of Escherichia coli heat-labile

enterotoxin (Etx) is a potent immunogen in vivo and has profound modulatory effects on **EtxB**-primed lymphocytes in vitro, properties which are dependent on its ability to bind to GM1 ganglioside receptors. Here, it is shown that cross-linking GM1 by **EtxB** causes a differential effect on mature CD4(+) and CD8(+) T cells from lymph node cultures proliferating in response to an unrelated antigen, **ovalbumin**. Addition of **EtxB** to such cultures led to the complete depletion of CD8(+) T cells compared with enhanced activation of CD4(+) cells [as measured by expression of CD25 (IL-2Ralpha)]. By contrast, addition of a mutant **EtxB**, **EtxB** (G33D), which does not bind to GM1, failed to trigger CD8(+) T cell depletion. When **EtxB** was added to isolated non-immune CD8(+) lymphocytes rapid (12-18 h) alterations in nuclear morphology and the appearance of sub-G0/G1 levels of DNA were induced; properties which are characteristic of cells undergoing apoptosis. **EtxB** (G33D) failed to trigger apoptosis, indicating that the induction of the apoptotic signal was dependent on the binding of GM1. These findings provide an insight into the potent immunogenicity and immunomodulatory properties of *E. coli* enterotoxins as well as heralding a novel method for the selective induction of apoptosis in mature CD8(+) T lymphocytes.

=> s williams n?/au

L18 5961 WILLIAMS N?/AU

=> s l18 and "IL-10"

L19 20 L18 AND "IL-10"

=> s l19 and "EtxB"

L20 3 L19 AND "ETXB"

=> dup remove l20

PROCESSING COMPLETED FOR L20

L21 3 DUP REMOVE L20 (0 DUPLICATES REMOVED)

=> d l21 1-3 cbib abs

L21 ANSWER 1 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS
2001:82983 Document No.: PREV200100082983. Nasal administration of admixed *E. coli* heat-labile toxin B subunit (**EtxB**) and insulin prevents autoimmune diabetes mellitus (IDDM) in NOD mice by inducing regulatory CD4+ cells. Turcanu, Victor (1); Williams, Neil A. (1). (1) Dept. Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD UK. Immunology, (December, 2000) Vol. 101, No. Supplement 1, pp. 59. print. Meeting Info.: Annual Congress of the British Society for Immunology Harrogate, UK December 05-08, 2000 British Society for Immunology. ISSN: 0019-2805. Language: English. Summary Language: English.

L21 ANSWER 2 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS
2001:82982 Document No.: PREV200100082982. Immunomodulation of the human MLR by *E. coli*-heat-labile toxin B subunit (**EtxB**): Induction of regulatory T cells. Turcanu, Victor (1); Williams, Neil A. (1). (1) Dept. Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD UK. Immunology, (December, 2000) Vol. 101, No. Supplement 1, pp. 59. print. Meeting Info.: Annual Congress of the British Society for Immunology Harrogate, UK December 05-08, 2000 British Society for Immunology. ISSN: 0019-2805. Language: English. Summary Language: English.

L21 ANSWER 3 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS
2000:138014 Document No.: PREV2000000138014. **EtxB** induces IL
-10 production by human monocytes and has immunomodulating
effects upon the mixed lymphocyte reaction. Turcanu, V. (1); Heaton, C.

P. E. (1); **Williams, N. A.** (1). (1) Department of Pathology and
Microbiology, University of Bristol, Bristol, BS8 1TD UK. Immunology.,
(Dec., 1999) Vol. 98, No. suppl. 1, pp. 36. Meeting Info.: Joint Congress
of the British Society for Immunology and the British Society for Allergy
& Clinical Immunology. Harrogate, England, UK November 30-December 03,
1999 British Society for Allergy & Clinical Immunology. ISSN: 0019-2805.
Language: English. Summary Language: English.

=>

---Logging off of STN---

=>

Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS

SINCE FILE

TOTAL

GHARAKHANIAN E	1990	178	62	VIROLOGY
GRIFFITH J P	1992	355	652	NATURE
GROMEIER M	1990	64	3590	J VIROL
GUTTMAN N	1977	82	25	VIROLOGY
HARLOW E	1988			ANTIBODIES LABORATOR
HARRISON S C	1990		37	VIROLOGY
KIRCHHAUSEN T	1983	80	2481	P NATL ACAD SCI USA
KRAUZEWICZ N	1990	64	4414	J VIROL
LAEMMLI U K	1970	227	680	NATURE
LEAVITT A D	1985	260	12803	J BIOL CHEM
LIDDINGTON R C	1991	354	278	NATURE
LIN W	1984	50	363	J VIROL
MCKENNA R	1992	355	137	NATURE
MONTROSS L	1991	65	4991	J VIROL
MORELAND R B	1991	65	1168	J VIROL
MOSCUFO N	1993	67	5075	J VIROL
NIBERT M L	1991	65	2372	J VIROL
PAUL A V	1987	84	7827	P NATL ACAD SCI USA
RAYMENT I	1982	295	110	NATURE
REINEMER P	1991	10	1997	EMBO J
RUECKERT R R	1991		409	FUNDAMENTAL VIROLOGY
SAHLI R	1993	192	142	VIROLOGY
SALUNKE D M	1989	56	88M	BIOPHYS J
SALUNKE D M	1986	46	895	CELL
SAMBROOK J	1989			MOL LONING LABORATOR
SCHMIDT M	1989	63	429	J VIROL
SIXMA T K	1991	351	371	NATURE
SIXMA T K	1992	355	561	NATURE
SMITH D B	1988	67	31	GENE
SMITH D B	1986	83	8703	P NATL ACAD SCI USA
STEHLE T				IN PRESS NATURE LOND
STREULI C H	1987	326	619	NATURE

=> d his

(FILE 'HOME' ENTERED AT 09:58:00 ON 02 MAR 2001)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 09:58:19 ON
02 MAR 2001

L1 36730 S ENTEROTOXIN
L2 5590 S L1 AND HEAT LABILE
L3 1 S L2 AND B SUBNIT
L4 649 S L2 AND MUTANT
L5 7 S L4 AND BINDING ASSAY
L6 3 DUP REMOVE L5 (4 DUPLICATES REMOVED)

=> s 14 and IL10

L7 0 L4 AND IL10

=> s 14 and reduce GM1 binding

L8 0 L4 AND REDUCE GM1 BINDING

=> dup remove 14

PROCESSING IS APPROXIMATELY 15% COMPLETE FOR L4
PROCESSING IS APPROXIMATELY 55% COMPLETE FOR L4
PROCESSING COMPLETED FOR L4
L9 234 DUP REMOVE L4 (415 DUPLICATES REMOVED)

=> s 19 and EtXB

L10 15 L9 AND ETXB

=> s 110 and cytokine

L11 1 L10 AND CYTOKINE

=> d 111

L11 ANSWER 1 OF 1 MEDLINE
AN 96133910 MEDLINE
DN 96133910
TI Potent immunogenicity of the B subunits of Escherichia coli **heat-labile enterotoxin**: receptor binding is essential and induces differential modulation of lymphocyte subsets.
AU Nashar T O; Webb H M; Eaglestone S; Williams N A; Hirst T R
CS Research School of Biosciences, University of Kent, Canterbury, Great Britain.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Jan 9) 93 (1) 226-30.
Journal code: PV3. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199604

=> dup remove 110

PROCESSING COMPLETED FOR L10
L12 15 DUP REMOVE L10 (0 DUPLICATES REMOVED)

=> d 112

L12 ANSWER 1 OF 15 MEDLINE
AN 1999134317 MEDLINE
DN 99134317
TI Structural basis for the differential toxicity of cholera toxin and Escherichia coli **heat-labile enterotoxin**. Construction of hybrid toxins identifies the A2-domain as the determinant of differential toxicity.
AU Rodighiero C; Aman A T; Kenny M J; Moss J; Lencer W I; Hirst T R
CS Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, United Kingdom.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Feb 12) 274 (7) 3962-9.
Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)

LA English
FS Priority Journals; Cancer Journals
EM 199905
EW 19990502

=> d 110 all 1-15

L10 ANSWER 1 OF 15 MEDLINE
AN 1999134317 MEDLINE
DN 99134317
TI Structural basis for the differential toxicity of cholera toxin and
Escherichia coli **heat-labile enterotoxin**.
Construction of hybrid toxins identifies the A2-domain as the determinant
of differential toxicity.
AU Rodighiero C; Aman A T; Kenny M J; Moss J; Lencer W I; Hirst T R
CS Department of Pathology and Microbiology, University of Bristol, School
of
Medical Sciences, Bristol BS8 1TD, United Kingdom.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Feb 12) 274 (7) 3962-9.
Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199905
EW 19990502
AB Cholera toxin (Ctx) and E. coli **heat-labile
enterotoxin** (Etx) are structurally and functionally similar AB5
toxins with over 80% sequence identity. When their action in polarized
human epithelial (T84) cells was monitored by measuring toxin-induced Cl-
ion secretion, Ctx was found to be the more potent of the two toxins.
Here, we examine the structural basis for this difference in toxicity by
engineering a set of **mutant** and hybrid toxins and testing their
activity in T84 cells. This revealed that the differential toxicity of
Ctx
and Etx was (i) not due to differences in the A-subunit's C-terminal KDEL
targeting motif (which is RDEL in Etx), as a KDEL to RDEL substitution
had
no effect on cholera toxin activity; (ii) not attributable to the
enzymatically active A1-fragment, as hybrid toxins in which the
A1-fragment in Ctx was substituted for that of Etx (and vice versa) did
not alter relative toxicity; and (iii) not due to the B-subunit, as the
replacement of the B-subunit in Ctx for that of Etx caused no alteration
in toxicity, thus excluding the possibility that the broader receptor
specificity of **EtxB** is responsible for reduced activity.
Remarkably, the difference in toxicity could be mapped to a 10-amino acid
segment of the A2-fragment that penetrates the central pore of the
B-subunit pentamer. A comparison of the in vitro stability of two hybrid
toxins, differing only in this 10-amino acid segment, revealed that the
Ctx A2-segment conferred a greater stability to the interaction between
the A- and B-subunits than the corresponding segment from Etx A2. This
suggests that the reason for the relative potency of Ctx compared with
Etx
stems from the increased ability of the A2-fragment of Ctx to maintain
holotoxin stability during uptake and transport into intestinal
epithelia.

CT Check Tags: Human; Support, Non-U.S. Gov't
 Amino Acid Sequence
 *Bacterial Toxins: TO, toxicity
 Cell Line
 *Cholera Toxin: TO, toxicity
 Electrophoresis, Polyacrylamide Gel
 *Enterotoxins: TO, toxicity
 Molecular Sequence Data
 Peptide Fragments: TO, toxicity
 Polymerase Chain Reaction

RN 9012-63-9 (Cholera Toxin)
 CN 0 (enterotoxin LT); 0 (Bacterial Toxins); 0 (Enterotoxins); 0 (Peptide Fragments)

L10 ANSWER 2 OF 15 MEDLINE
 AN 1998018503 MEDLINE
 DN 98018503
 TI Modulation of B-cell activation by the B subunit of Escherichia coli enterotoxin: receptor interaction up-regulates MHC class II, B7, CD40, CD25 and ICAM-1.

AU Nashar T O; Hirst T R; Williams N A
 CS School of Medical Sciences, University of Bristol, UK.
 SO IMMUNOLOGY, (1997 Aug) 91 (4) 572-8.
 Journal code: GH7. ISSN: 0019-2805.
 CY ENGLAND: United Kingdom
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals; Cancer Journals
 EM 199801
 EW 19980104
 AB The B subunits of cholera toxin (CtxB) and Escherichia coli heat-labile enterotoxin (EtxB) are non-toxic lectins that bind and cross-link a ubiquitous cell glycolipid receptor, ganglioside GM1, and are recognized as potent mucosal and systemic immunogens. Here we examine the role of EtxB receptor occupancy in modulating the activation of B cells, in vitro, in primary lymphocyte cultures containing B and T cells. When 48-hr spleen cell cultures containing EtxB were compared with those in the presence of a non-receptor binding mutant, EtxB(G33D), a marked shift in the ratio of CD4+ T cells: B cells was noted. Evidence suggested that this was the result of either enhanced survival or proliferation of

B cells associated with receptor occupancy by EtxB. Investigation revealed that EtxB induced only a minimal increase in proliferation above that of EtxB(G33D), in mixed cell cultures, and failed to induce any cell division of purified B cells or T cells. In contrast, receptor-binding by EtxB markedly up-regulated the expression of major histocompatibility complex (MHC) class II, B7, intracellular adhesion molecule-1 (ICAM-1), CD40 and CD25 on the B-cell surface. These results indicate that the polyclonal effects of EtxB on B cells are not associated with wide-scale proliferation, but more likely with maintenance of B-cell survival by activation of molecules essential for B-cell differentiation. The findings also highlight the essential role of GM1-interaction with EtxB in the regulation of lymphocyte responses.

CT Check Tags: Animal; Female; Support, Non-U.S. Gov't
 Antigens, CD40: ME, metabolism
 Antigens, CD80: ME, metabolism

*B-Lymphocytes: IM, immunology
 *Bacterial Toxins: IM, immunology
 Cell Culture
 Cell Division: IM, immunology
 CD4-Positive T-Lymphocytes: IM, immunology
 *Enterotoxins: IM, immunology
 *Guanylate Cyclase: IM, immunology
 Histocompatibility Antigens Class II: ME, metabolism
 Intercellular Adhesion Molecule-1: ME, metabolism
 *Lymphocyte Transformation: IM, immunology
 Mice
 Mice, Inbred BALB C
 Receptors, Interleukin-2: ME, metabolism
 *Receptors, Peptide: IM, immunology
 Up-Regulation (Physiology): IM, immunology
 126547-89-5 (Intercellular Adhesion Molecule-1)
 EC 4.6.1.2 (Guanylate Cyclase); 0 (**enterotoxin** receptor); 0 (
enterotoxin LT); 0 (Antigens, CD40); 0 (Antigens, CD80); 0
 (Bacterial Toxins); 0 (**Enterotoxins**); 0 (Histocompatibility
 Antigens Class II); 0 (Receptors, Interleukin-2); 0 (Receptors, Peptide)

L10 ANSWER 3 OF 15 MEDLINE
 AN 97289759 MEDLINE
 DN 97289759
 TI Prevention of autoimmune disease due to lymphocyte modulation by the
 B-subunit of Escherichia coli **heat-labile**
enterotoxin.
 AU Williams N A; Stasiuk L M; Nashar T O; Richards C M; Lang A K; Day M J;
 Hirst T R
 CS Department of Pathology and Microbiology, School of Medical Sciences,
 University of Bristol, Bristol BS8 1TD, United Kingdom.
 SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF
 AMERICA, (1997 May 13) 94 (10) 5290-5.
 Journal code: PV3. ISSN: 0027-8424.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals; Cancer Journals
 EM 199708
 EW 19970801
 AB We demonstrate that the receptor binding moiety of Escherichia coli
heat-labile enterotoxin (EtxB) can
 completely prevent autoimmune disease in a murine model of arthritis.
 Injection of male DBA/1 mice at the base of the tail with type II
 collagen
 in the presence of complete Freund's adjuvant normally leads to
 arthritis,
 as evidenced by inflammatory infiltration and swelling of the joints. A
 separate injection of **EtxB** at the same time as collagen
 challenge prevented leukocyte infiltration, synovial hyperplasia, and
 degeneration of the articular cartilage and reduced clinical symptoms of
 disease by 82%. The principle biological property of **EtxB** is its
 ability to bind to the ubiquitous cell surface receptor GM1 ganglioside,
 and to other galactose-containing glycolipids and galactoproteins. The
 importance of receptor interaction in mediating protection from arthritis
 was demonstrated by the failure of a non-receptor-binding **mutant**
 of **EtxB** to elicit any protective effect. Analysis of T cell
 responses to collagen, in cultures of draining lymph node cells, revealed

that protection was associated with a marked increase in interleukin 4 production concomitant with a reduction in interferon gamma levels. Furthermore, in protected mice there was a significant reduction in anti-collagen antibody levels as well as an increase in the IgG1/IgG2a ratio. These observations show that protection is associated with a shift in the Th1/Th2 balance as well as a general reduction in the extent of

the anti-type II collagen immune response. This suggests that **EtxB**-receptor-mediated modulation of lymphocyte responses provides a means of preventing autoimmune disease.

CT Check Tags: Animal; Male; Support, Non-U.S. Gov't

Antibody Formation

Arthritis, Adjuvant: IM, immunology

Arthritis, Adjuvant: PA, pathology

*Arthritis, Adjuvant: PC, prevention & control

Autoimmune Diseases: IM, immunology

Autoimmune Diseases: PA, pathology

*Autoimmune Diseases: PC, prevention & control

*Bacterial Toxins: TU, therapeutic use

Collagen

*Enterotoxins: TU, therapeutic use

Escherichia coli

G(M1) Ganglioside: IM, immunology

G(M1) Ganglioside: ME, metabolism

Interferon Type II: BI, biosynthesis

Interleukin-4: BI, biosynthesis

Lymphocyte Transformation

Mice

Mice, Inbred DBA

Protein Binding

Receptors, Cell Surface: IM, immunology

T-Lymphocytes: DE, drug effects

*T-Lymphocytes: IM, immunology

RN 37758-47-7 (G(M1) Ganglioside); 82115-62-6 (Interferon Type II);
9007-34-5

(Collagen)

CN 0 (enterotoxin LT); 0 (Bacterial Toxins); 0 (Enterotoxins); 0 (Interleukin-4); 0 (Receptors, Cell Surface)

L10 ANSWER 4 OF 15 MEDLINE

AN 97128619 MEDLINE

DN 97128619

TI A pH-dependent conformational change in the B-subunit pentamer of Escherichia coli **heat-labile enterotoxin**: structural basis and possible functional role for a conserved feature of the AB5 toxin family.

AU Ruddock L W; Webb H M; Ruston S P; Cheesman C; Freedman R B; Hirst T R
CS Research School of Biosciences, University of Kent at Canterbury, U.K..
l.w.ruddock@ukc.ac.uk

SO BIOCHEMISTRY, (1996 Dec 17) 35 (50) 16069-76.
Journal code: A0G. ISSN: 0006-2960.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199703

AB The non-covalently associated B-subunit moieties of AB5 toxins, such as cholera toxin and related diarrheagenic **enterotoxins**, exhibit

exceptional pH stability and remain pentameric at pH values as low as 2.0.

Here, we investigate the structural basis of a pH-dependent conformational

change which occurs within the B5 structure of *Escherichia coli* heat-labile enterotoxin (EtxB) at

around pH 5.0. The use of far-UV CD and fluorescence spectroscopy showed that EtxB pentamers undergo a fully reversible pH-dependent conformational change with a pKa of 4.9 +/- 0.1 (R2 = 0.999) or 5.13 +/- 0.01 (R2 = 0.999), respectively. This renders the pentamer susceptible to SDS-mediated disassembly and decreases its thermal stability by 18

degrees

C. A comparison of the pH-dependence of the structural change in EtxB5, with that of a mutant containing a Ser substitution at His 57, revealed that the pKa of the conformational change was shifted from ca. 5.1 to 4.4. This finding suggests that protonation of the imidazole side chain of His 57 might facilitate disruption of a spatially adjacent salt bridge, located between Glu 51 and Lys 91 in each B-subunit, thus triggering the conformational change in the pentameric structure. The pH-dependent conformational change was found to be inhibited when B-subunits bound to monosialoganglioside, GM1; and to have no effect on the stability of interaction between A- and B-subunits within the AB5 complex. This suggests that the conformational change is unlikely to have a direct involvement in toxicity. Conservation of the pH-dependent conformational change in the AB5 toxin family, combined with the

potential

exposure of the hydrophobic core of beta-barrel in the monomeric units, leads to the proposal that the conformational change may be the common feature that ensures the secretion of these proteins from the Vibrionaceae.

CT Check Tags: Animal; Comparative Study; Human; Support, Non-U.S. Gov't
Amino Acid Sequence

*Bacterial Toxins: CH, chemistry

Bacterial Toxins: IP, isolation & purification

Cholera Toxin: CH, chemistry

Conserved Sequence

Electrophoresis, Polyacrylamide Gel

*Enterotoxins: CH, chemistry

Enterotoxins: IP, isolation & purification

Escherichia coli

Histidine

Hydrogen-Ion Concentration

Kinetics

Lysine

Macromolecular Systems

Models, Molecular

*Protein Conformation

Swine

Tryptophan

RN 56-87-1 (Lysine); 7006-35-1 (Histidine); 73-22-3 (Tryptophan); 9012-63-9 (Cholera Toxin)

CN 0 (enterotoxin LT); 0 (Bacterial Toxins); 0 (Enterotoxins); 0 (Macromolecular Systems)

L10 ANSWER 5 OF 15 MEDLINE

AN 96324796 MEDLINE

DN 96324796

TI Cross-linking of cell surface ganglioside GM1 induces the selective

apoptosis of mature CD8+ T lymphocytes.

AU Nahar T O; Williams N A; Hirst T R
 CS Research School of Biosciences, University of Kent, Canterbury, UK.
 SO INTERNATIONAL IMMUNOLOGY, (1996 May) 8 (5) 731-6. Ref: 24
 Journal code: AY5. ISSN: 0953-8178.
 CY ENGLAND: United Kingdom
 DT Journal; Article; (JOURNAL ARTICLE)
 General Review; (REVIEW)
 (REVIEW, TUTORIAL)
 LA English
 FS Priority Journals
 EM 199705
 EW 19970505
 AB Gangliosides are glycosphingolipids found ubiquitously on the surface of mammalian cells. They contain a ceramide tail that is inserted into the membrane and exposed carbohydrate and sialic acid moieties. The non-toxic B subunit oligomer (**EtxB**) of Escherichia coli **heat-labile enterotoxin** (Etx) is a potent immunogen in vivo and has profound modulatory effects on **EtxB**-primed lymphocytes in vitro, properties which are dependent on its ability to bind to GM1 ganglioside receptors. Here, it is shown that cross-linking GM1 by **EtxB** causes a differential effect on mature CD4(+) and CD8(+) T cells from lymph node cultures proliferating in response to an unrelated antigen, ovalbumin. Addition of **EtxB** to such cultures led to the complete depletion of CD8(+) T cells compared with enhanced activation of CD4(+) cells [as measured by expression of CD25 (IL-2Ralpha)]. By contrast, addition of a **mutant EtxB, EtxB** (G33D), which does not bind to GM1, failed to trigger CD8(+) T cell depletion. When **EtxB** was added to isolated non-immune CD8(+) lymphocytes rapid (12-18 h) alterations in nuclear morphology and the appearance of sub-G0/G1 levels of DNA were induced; properties which are characteristic of cells undergoing apoptosis. **EtxB**(G33D) failed to trigger apoptosis, indicating that the induction of the apoptotic signal was dependent on the binding of GM1. These findings provide an insight into the potent immunogenicity and immunomodulatory properties of E. coli **enterotoxins** as well as heralding a novel method for the selective induction of apoptosis in mature CD8(+) T lymphocytes.

CT Check Tags: Animal; Support, Non-U.S. Gov't
 Apoptosis: DE, drug effects
 *Apoptosis: IM, immunology
 *Cross-Linking Reagents: ME, metabolism
 Cross-Linking Reagents: PD, pharmacology
 *CD8-Positive T-Lymphocytes: DE, drug effects
 *G(M1) Ganglioside: IM, immunology
 *G(M1) Ganglioside: ME, metabolism
 G(M1) Ganglioside: PD, pharmacology
 *Membrane Lipids: IM, immunology
 *Membrane Lipids: ME, metabolism
 Membrane Lipids: PD, pharmacology

RN 37758-47-7 (G(M1) Ganglioside)
 CN 0 (Cross-Linking Reagents); 0 (Membrane Lipids)

L10 ANSWER 6 OF 15 MEDLINE
 AN 96133910 MEDLINE
 DN 96133910
 TI Potent immunogenicity of the B subunits of Escherichia coli **heat-labile enterotoxin**: receptor binding is essential and induces differential modulation of lymphocyte subsets.

AU Nashar T O; Webb H M; Eaglestone S; Williams N A; Hirst T R
CS Research School of Biosciences, University of Kent, Canterbury, Great Britain.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Jan 9) 93 (1) 226-30.
Journal code: PV3. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199604
AB The importance of receptor binding in the potent immunogenicity of *Escherichia coli* **heat-labile enterotoxin B** subunit (**EtxB**) was tested by comparing its immunological properties with those of a receptor binding mutant, **EtxB** (G33D). Subcutaneous immunization of **EtxB**(G33D) resulted in 160-fold reduction in antibody titer compared with wild-type **EtxB**, whereas its oral delivery failed to provoke any detectable secretory or serum anti-B subunit responses. Moreover, the two proteins induced strikingly different effects on lymphocyte cultures in vitro. **EtxB**, in comparison with **EtxB**(G33D), caused an increase in the proportion of B cells, many of which were activated (CD25+); the complete depletion of CD8+ T cells; an increase in the activation of CD4+ T cells; and an increase in interleukin 2 and a decrease in interferon gamma.

These data indicate that **EtxB** exerts profound effects on immune cells, suggesting that its potent immunogenicity is dependent not only on efficient receptor-mediated uptake, but also on direct receptor-mediated immunomodulation of lymphocyte subsets.

CT Check Tags: Animal; Support, Non-U.S. Gov't
Adjuvants, Immunologic
Antibodies, Bacterial: IM, immunology
Bacterial Toxins: CH, chemistry
*Bacterial Toxins: IM, immunology
Bacterial Toxins: ME, metabolism
Base Sequence
Cytokines: BI, biosynthesis
DNA Primers: CH, chemistry
Enterotoxins: CH, chemistry
*Enterotoxins: IM, immunology
Enterotoxins: ME, metabolism
*Escherichia coli: IM, immunology
*G(M1) Ganglioside: PH, physiology
Immunologic Capping
Immunophenotyping
*Lymphocyte Subsets: IM, immunology
Lymphocyte Transformation
Mice
Mice, Inbred BALB C
Molecular Sequence Data
Mutagenesis, Site-Directed
Receptors, Cell Surface: ME, metabolism
Structure-Activity Relationship
RN 37758-47-7 (G(M1) Ganglioside)
CN 0 (enterotoxin LT); 0 (Adjuvants, Immunologic); 0 (Antibodies, Bacterial); 0 (Bacterial Toxins); 0 (Cytokines); 0 (DNA Primers); 0 (Enterotoxins); 0 (Receptors, Cell Surface)

L10 ANSWER 7 OF 15 MEDLINE
 AN 96102052 MEDLINE
 DN 96102052
 TI Kinetics of acid-mediated disassembly of the B subunit pentamer of
 Escherichia coli **heat-labile enterotoxin**.
 Molecular basis of pH stability.
 AU Ruddock L W; Ruston S P; Kelly S M; Price N C; Freedman R B; Hirst T R
 CS Biological Laboratory, University of Kent, Canterbury, United Kingdom.
 SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Dec 15) 270 (50) 29953-8.
 Journal code: HIV. ISSN: 0021-9258.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals; Cancer Journals
 EM 199604
 AB The B-subunit pentamer of Escherichia coli **heat-labile enterotoxin (EtxB)** is highly stable, maintaining its quaternary structure in a range of conditions that would normally be expected to cause protein denaturation. In this paper the structural stability of **EtxB** has been studied as a function of pH by electrophoretic, immunochemical, and spectroscopic techniques.
 Disassembly of the cyclic pentameric structure of human **EtxB** occurs only below pH 2. As determined by changes in intrinsic fluorescence this process follows first-order kinetics, with the rate constant for disassembly being proportional to the square of the H⁺ ion concentration, and with an activation energy of 155 kJ mol⁻¹. A C-terminal deletion mutant, hEtxB214, similarly shows first-order kinetics for disassembly but with a higher pH threshold, resulting in disassembly being seen at pH 3.4 and below. These findings are consistent with the rate-limiting step for disassembly of human **EtxB** being the simultaneous disruption of two interfaces by protonation of two C-terminal carboxylates. By comparison, disassembly of the B-subunit of cholera toxin (CtxB), a protein which shows 80% sequence identity with **EtxB**, exhibits a much lower stability to acid conditions; with disassembly of CtxB occurring below pH 3.9, with an activation energy of 81 kJ mol⁻¹. Reasons for the observed differences in acid stability are discussed, and the implications of these findings to the development of oral vaccines using **EtxB** and CtxB are considered.
 CT Check Tags: Comparative Study; Human; Support, Non-U.S. Gov't
 *Bacterial Toxins: CH, chemistry
 Bacterial Toxins: IP, isolation & purification
 *Bacterial Toxins: ME, metabolism
 Drug Stability
 Electrophoresis, Polyacrylamide Gel
 *Enterotoxins: CH, chemistry
 Enterotoxins: IP, isolation & purification
 *Enterotoxins: ME, metabolism
 *Escherichia coli
 Escherichia coli: IP, isolation & purification
 Hydrogen-Ion Concentration
 Kinetics
 Models, Structural
 Spectrophotometry, Ultraviolet
 Thermodynamics

CN 0 (enterotoxin LT); 0 (Bacterial Toxins); 0 (Enterotoxins)

L10 ANSWER 8 OF 15 MEDLINE

AN 95058206 MEDLINE

DN 95058206

TI Suppression of temperature-sensitive assembly **mutants** of **heat-labile enterotoxin B** subunits.

AU Sandkvist M; Bagdasarian M

CS Department of Microbiology, Michigan State University, East Lansing 48824..

SO MOLECULAR MICROBIOLOGY, (1993 Nov) 10 (3) 635-45.

Journal code: MOM. ISSN: 0950-382X.

CY ENGLAND: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199502

AB Deletions or substitutions of amino acids at the carboxyl-terminus of the

heat-labile enterotoxin B subunit (**EtxB**)

affect its assembly into pentamers in a temperature-dependent manner. At 42 degrees C, the mutations prevent the B subunits from achieving their final pentameric structure resulting in membrane association of the monomers. However, **mutant** B subunits produced at 30 degrees C assemble, in the periplasm, into pentamers that remain stable when transferred to 42 degrees C, indicating that the **mutant** pentamers are stable under conditions where their formation is inhibited. The **mutant** pentamers are, similarly to wild-type pentamers, SDS-resistant and stable, in vitro, at temperatures up to 65 degrees C. This suggests that although the C-terminal amino acids are

part

of the subunit interface, they appear not to contribute significantly to the stability of the final pentameric complex, but are instead essential for the formation or stabilization of an assembly intermediate in the pentamerization process. Single second site mutations suppress the assembly defect of **mutant** EtxB191.5, which carries substitutions at its C-terminus. The Thr-->Ile replacement at position 75 in the alpha 2-helix probably restores the van der Waals contact between residues 75 and 101, which had been greatly reduced by the Met-->Leu substitution at position 101 in the beta 6-strand of EtxB191.5. Interaction between the alpha 2-helix and beta 6-strand which contains the C-terminus probably stabilizes a conformation essential for assembly and is therefore

required

for the formation of pentamers.

CT Check Tags: Support, Non-U.S. Gov't
Amino Acid Sequence

*Bacterial Toxins: GE, genetics

Bacterial Toxins: ME, metabolism

*Enterotoxins: GE, genetics

Enterotoxins: ME, metabolism

*Escherichia coli: GE, genetics

Molecular Sequence Data

Protein Conformation

*Protein Folding

Sequence Deletion

Spheroplasts: ME, metabolism

*Suppression, Genetic

Temperature

CN 0 (**enterotoxin** LT); 0 (Bacterial Toxins); 0 (**Enterotoxins**)
 GEN **etxB**

L10 ANSWER 9 OF 15 MEDLINE
 AN 93101683 MEDLINE
 DN 93101683
 TI Intermolecular interactions between the A and B subunits of **heat-labile enterotoxin** from *Escherichia coli* promote holotoxin assembly and stability in vivo.
 AU Streatfield S J; Sandkvist M; Sixma T K; Bagdasarian M; Hol W G; Hirst T
 R
 CS Biological Laboratory, University of Kent, Canterbury, Great Britain..
 SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1992 Dec 15) 89 (24) 12140-4.
 Journal code: PV3. ISSN: 0027-8424.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals; Cancer Journals
 EM 199303
 AB Cholera toxin and the related **heat-labile enterotoxin** (LT) produced by *Escherichia coli* consist of a holotoxin of one A subunit and five B subunits (AB5). Here we investigate the domains of the A subunit (EtxA) of *E. coli* LT which influence the events of B-subunit (**EtxB**) oligomerization and the formation of a stable AB5 holotoxin complex. We show that the C-terminal 14 amino acids of the A subunit comprise two functional domains that differentially affect oligomerization and holotoxin stability. Deletion of the last 14 amino acids (-14) from the A subunit resulted in a molecule that was significantly impaired in its capacity to promote the assembly of a **mutant** B subunit, EtxB191.5. In contrast, deletion of the last four amino acids (-4) from the A subunit gave a molecule that retained such a capacity. This suggests that C-terminal residues within the -14 to -4 region of the A subunit are important for promoting the oligomerization of **EtxB**. In addition, we demonstrate that the truncated A subunit lacking the last 4 amino acids was unable to form a stable AB5 holotoxin complex even though it promoted B-subunit oligomerization. This suggests that the last 4 residues of the A subunit function as an "anchoring" sequence responsible for maintaining the stability of A/B subunit interaction during holotoxin assembly. These data represent an important example of how intermolecular interactions between polypeptides in vivo can modulate the folding and assembly of a macromolecular complex.

CT Check Tags: Support, Non-U.S. Gov't
 Amino Acid Sequence
 *Bacterial Toxins: CH, chemistry
 Bacterial Toxins: GE, genetics
 Bacterial Toxins: ME, metabolism
 Base Sequence
 *Enterotoxins: CH, chemistry
 Enterotoxins: GE, genetics
 Enterotoxins: ME, metabolism
 *Escherichia coli: ME, metabolism
 Gene Expression Regulation, Bacterial
 Genes, Structural, Bacterial

Macromolecular Systems
 Molecular Sequence Data
 Mutagenesis, Site-Directed
 Protein Conformation
 Protein Denaturation
 Recombinant Proteins
 Regulatory Sequences, Nucleic Acid
 RNA, Messenger: GE, genetics
 Structure-Activity Relationship
 Transcription, Genetic
 Translation, Genetic

CN 0 (**enterotoxin** LT); 0 (Bacterial Toxins); 0 (**Enterotoxins**); 0 (Macromolecular Systems); 0 (Recombinant Proteins); 0 (RNA, Messenger)

GEN etxA; **etxB**

L10 ANSWER 10 OF 15 MEDLINE
 AN 92374846 MEDLINE
 DN 92374846
 TI A homologue of the Escherichia coli DsbA protein involved in disulphide bond formation is required for **enterotoxin** biogenesis in Vibrio cholerae.
 AU Yu J; Webb H; Hirst T R
 CS Biological Laboratory, University of Kent, Canterbury, UK.
 SO MOLECULAR MICROBIOLOGY, (1992 Jul) 6 (14) 1949-58.
 Journal code: MOM. ISSN: 0950-382X.
 CY ENGLAND: United Kingdom
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 OS GENBANK-X64725
 EM 199211
 AB A strain of Vibrio cholerae, which had been engineered to express high levels of the non-toxic B subunit (**EtxB**) of Escherichia coli **heat-labile enterotoxin**, was subjected to transposon (TnphoA) mutagenesis. Two chromosomal TnphoA insertion mutations of the strain were isolated that showed a severe defect in the amount of **EtxB** produced. The loci disrupted by TnphoA in the two **mutant** derivatives were cloned and sequenced, and this revealed that the transposon had inserted at different sites in the same gene. The open reading frame of the gene predicts a 200-amino-acid exported protein, with a Cys-X-X-Cys motif characteristic of thioredoxin, protein disulphide isomerase, and DsbA (a periplasmic protein required for disulphide bond formation in E. coli). The V. cholerae protein exhibited 40% identity with the DsbA protein of E. coli, including 90% identity in the region of the active-site motif. Introduction of a plasmid encoding E. coli DsbA into the V. cholerae TnphoA derivatives was found to restore **enterotoxin** formation, whilst expression of Etx or **EtxB** in a dsbA **mutant** of E. coli confirmed that DsbA is required for **enterotoxin** formation in E. coli. These results suggest that, since each **EtxB** subunit contains a single intramolecular disulphide bond, a transient intermolecular interaction with DsbA occurs during toxin subunit folding which catalyses formation of the disulphide in vivo.

CT Check Tags: Support, Non-U.S. Gov't

Amino Acid Sequence
 *Bacterial Proteins: GE, genetics
 Bacterial Toxins: BI, biosynthesis
 *Bacterial Toxins: GE, genetics
 Base Sequence
 Disulfides: ME, metabolism
 DNA Transposable Elements: PH, physiology
Enterotoxins: BI, biosynthesis
 *Enterotoxins: GE, genetics
 Escherichia coli: CH, chemistry
 *Escherichia coli: GE, genetics
 Genes, Bacterial: GE, genetics
 *Genes, Bacterial: PH, physiology
 *Isomerases: GE, genetics
 Molecular Sequence Data
 Mutagenesis, Insertional: GE, genetics
 Recombinant Proteins: BI, biosynthesis
 Sequence Homology, Nucleic Acid
 Vibrio cholerae: CH, chemistry
 *Vibrio cholerae: GE, genetics
 CN EC 5. (Isomerases); EC 5.3.4.1 (Protein Disulfide-Isomerase); 0 (**enterotoxin** LT); 0 (Bacterial Proteins); 0 (Bacterial Toxins); 0 (Disulfides); 0 (DNA Transposable Elements); 0 (**Enterotoxins**); 0 (Recombinant Proteins)

L10 ANSWER 11 OF 15 MEDLINE
 AN 92268852 MEDLINE
 DN 92268852
 TI Expression of the B subunit of Escherichia coli **heat-labile enterotoxin** in a marine Vibrio and in a **mutant** that is pleiotropically defective in the secretion of extracellular proteins.
 AU Leece R; Hirst T R
 CS Department of Genetics, University of Leicester, UK..
 SO JOURNAL OF GENERAL MICROBIOLOGY, (1992 Apr) 138 (Pt 4) 719-24.
 Journal code: I87. ISSN: 0022-1287.
 CY ENGLAND: United Kingdom
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199208
 AB A marine Vibrio (designated Vibrio sp. 60) that is related to Vibrio anguillarum was used as a host for a plasmid that encodes the non-toxic B subunit (**EtxB**) of Escherichia coli **heat-labile enterotoxin**. Expression of **EtxB** in Vibrio sp. 60 resulted in the efficient and selective secretion of the B subunit into the extracellular growth medium. This indicated that Vibrio sp. 60, which does not normally produce cholera-like **enterotoxins**, nonetheless possesses a secretory machinery that permits these toxins to be translocated across its cytoplasmic and outer membranes. Expression of **EtxB** in a sec **mutant** of Vibrio sp. 60 (MVT1192), which had previously been shown to be defective in the secretion of several extracellular proteins, resulted in approximately 95% of the B subunit remaining entrapped within the periplasm of the bacterial cell envelope. This implies that the mutation in MVT1192 defines a locus that determines a common step in the secretion of extracellular proteins, including oligomeric toxins.
 CT Check Tags: Comparative Study; Support, Non-U.S. Gov't

*Bacterial Proteins: SE, secretion
 Bacterial Toxins: BI, biosynthesis
 *Bacterial Toxins: GE, genetics
 Culture Media
 Enterotoxins: BI, biosynthesis
 *Enterotoxins: GE, genetics
 *Escherichia coli: GE, genetics
 *Gene Expression
 Genetic Vectors
 *Mutation
 Phenotype
 Plasmids
 *Vibrio: GE, genetics
 Vibrio: ME, metabolism
 CN 0 (enterotoxin LT); 0 (Bacterial Proteins); 0 (Bacterial
 Toxins); 0 (Culture Media); 0 (Enterotoxins); 0 (Plasmids)
 GEN sec

 L10 ANSWER 12 OF 15 MEDLINE
 AN 92140031 MEDLINE
 DN 92140031
 TI Targeting and assembly of an oligomeric bacterial enterotoxoid in the
 endoplasmic reticulum of *Saccharomyces cerevisiae*.
 AU Schonberger O; Hirst T R; Pines O
 CS Department of Molecular Biology, Hebrew University, Hadassah Medical
 School, Jerusalem, Israel..
 SO MOLECULAR MICROBIOLOGY, (1991 Nov) 5 (11) 2663-71.
 Journal code: MOM. ISSN: 0950-382X.
 CY ENGLAND: United Kingdom
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199205
 AB A hybrid protein consisting of the *Escherichia coli* lipoprotein signal
 sequence attached to the mature sequence of the B subunit of **heat**
-labile enterotoxin (Lipo-**EtxB**) was
 expressed in yeast and *E. coli*. Analyses of cell lysates from
Saccharomyces cerevisiae and *E. coli* expressing the protein revealed that
 both organisms were able to assemble Lipo-**EtxB** into oligomers
 that were (i) stable in the presence of sodium dodecyl sulphate, (ii)
 resistant to proteinase K degradation, and (iii) able to bind to
 GM1-ganglioside receptors. Each of these properties are characteristic of
 the wild-type B subunit pentamer produced in *E. coli*. Assembly of Lipo-
EtxB was found to be unaffected in a *sec18* mutant of *S.*
cerevisiae, which possesses a temperature-sensitive defect in protein
 transport from the endoplasmic reticulum (ER) to the Golgi apparatus, but
 was found not to assemble in a *sec53* mutant, which causes the
 misfolding of proteins targeted to the ER. A *kar2-1* mutation with a
 defect
 in the yeast homologue of BiP caused an 18-fold reduction in Lipo-
EtxB assembly at the non-permissive temperature in *S. cerevisiae*.
 However, introduction of the wild-type *KAR2* gene on a plasmid into the
kar2-1 mutant completely suppressed the inhibition of Lipo-
EtxB assembly. This provides the first evidence that *KAR2*
 facilitates the assembly of an oligomeric protein in yeast and thus
 implicates *KAR2* as a 'molecular chaperone'. The possible mechanisms of
 enterotoxoid assembly in *E. coli* and *S. cerevisiae* are discussed.
 CT Check Tags: Comparative Study; Support, Non-U.S. Gov't

Amino Acid Sequence

*Bacterial Proteins: BI, biosynthesis
Bacterial Proteins: GE, genetics
*Bacterial Toxins: BI, biosynthesis
Bacterial Toxins: GE, genetics
*Endoplasmic Reticulum: ME, metabolism

*Enterotoxins: BI, biosynthesis

Enterotoxins: GE, genetics

*Escherichia coli: GE, genetics
Escherichia coli: ME, metabolism
Fungal Proteins: GE, genetics
Fungal Proteins: ME, metabolism
Golgi Apparatus: ME, metabolism

*Lipoproteins: BI, biosynthesis

Lipoproteins: GE, genetics

Molecular Sequence Data

Protein Processing, Post-Translational

*Recombinant Fusion Proteins: BI, biosynthesis

*Saccharomyces cerevisiae: ME, metabolism

Signal Peptides: GE, genetics

CN 0 (enterotoxin LT); 0 (Bacterial Proteins); 0 (Bacterial Toxins); 0 (Enterotoxins); 0 (Fungal Proteins); 0 (KAR2 protein, yeast); 0 (Lipoproteins); 0 (Recombinant Fusion Proteins); 0 (Signal Peptides); 0 (SEC18 protein); 0 (SEC53 protein)

L10 ANSWER 13 OF 15 MEDLINE

AN 90368708 MEDLINE

DN 90368708

TI Minimal deletion of amino acids from the carboxyl terminus of the B subunit of **heat-labile enterotoxin** causes defects in its assembly and release from the cytoplasmic membrane of *Escherichia coli*.

AU Sandkvist M; Hirst T R; Bagdasarian M

CS Department of Microbiology, Michigan State University, Lansing 48909..

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1990 Sep 5) 265 (25) 15239-44.
Journal code: HIV. ISSN: 0021-9258.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

EM 199012

AB Minimal alterations at the carboxyl terminus of the B subunit (

EtxB) of **heat-labile enterotoxin**

from *Escherichia coli* were found to have a marked effect on the assembly and release of this polypeptide into the periplasm. Nine **mutant EtxB** polypeptides were obtained by genetic manipulation of the 3'-end of the **etxB** gene using Bal31 nuclease digestion and codon substitution. A correlation was observed between the magnitude of the changes introduced at the carboxyl terminus and the extent to which the **mutant** polypeptides were defective in assembly and release. Some of the **mutant** B subunits, exemplified by those in which the last 2 amino acids had been deleted or in which the last 4 residues had been replaced by three different ones, were found to be only partially defective, with a proportion being associated with the periplasmic face

of

the cytoplasmic membrane and the remainder being exported to the periplasm. The portion associated with membranes was detected as monomers on sodium dodecyl sulfate-polyacrylamide gels, whereas the portion

exported to the periplasm were detected as assembled oligomers. We conclude that the last few amino acids at the carboxyl terminus of **EtxB** exert a profound influence on the assembly and release of the B subunit from the cytoplasmic membrane during export in *E. coli*.

CT Check Tags: Support, Non-U.S. Gov't
 Amino Acid Sequence
 Bacterial Toxins: BI, biosynthesis
 *Bacterial Toxins: GE, genetics
 Base Sequence
 Cell Membrane: ME, metabolism
 *Chromosome Deletion
 Codon: GE, genetics
 Enterotoxins: BI, biosynthesis
 *Enterotoxins: GE, genetics
 *Escherichia coli: GE, genetics
 Escherichia coli: ME, metabolism
 *Genes, Regulator
 Genetic Vectors
 Macromolecular Systems
 Molecular Sequence Data
 *Mutation
 *Terminator Regions (Genetics)
 Translation, Genetic

CN 0 (**enterotoxin** LT); 0 (Bacterial Toxins); 0 (Codon); 0 (**Enterotoxins**); 0 (Macromolecular Systems)

L10 ANSWER 14 OF 15 BIOSIS COPYRIGHT 2001 BIOSIS
 AN 1987:488218 BIOSIS
 DN BA84:122861
 TI ALTERATIONS AT THE CARBOXYL TERMINUS CHANGE ASSEMBLY AND SECRETION PROPERTIES OF THE B SUBUNIT OF ESCHERICHIA-COLI **HEAT-LABILE ENTEROTOXIN**.
 AU SANDKVIST M; HIRST T R; BAGDASARIAN M
 CS DEP. GENET., UNIV. LEICESTER, LEICESTER LE1 7RH, ENGLAND.
 SO J BACTERIOL, (1987) 169 (10), 4570-4576.
 CODEN: JOBAAY. ISSN: 0021-9193.
 FS BA; OLD
 LA English
 AB The gene encoding the B subunit of **heat-labile enterotoxin (etxB)** was mutated at its 3' end by targeted addition of random nucleotide sequences. Gene products from five mutated **etxB** genes, all of which were shown to encode B subunits with short carboxy-terminal amino acid extensions, were analyzed with respect to a range of functional and structural properties. One class of altered

B subunits, exemplified by EtxB124 and EtxB138, which both have seven extra amino acid residues, were found to be specifically defective in their ability to stably associate with A subunits and form holotoxin. Other altered B subunits were less subtly affected by extensions at their C termini and were, in addition to their failure to associate with A subunits, unable to translocate into the periplasm of *Escherichia coli*,

to pentamerize, or to bind to GM1 ganglioside. This suggests that the carboxy-terminal domain of **EtxB** mediates A subunit-B subunit interaction.

CC Biochemical Studies - Nucleic Acids, Purines and Pyrimidines 10062
 Biochemical Studies - Proteins, Peptides and Amino Acids *10064
 Biophysics - Molecular Properties and Macromolecules *10506

Metabolism - Proteins, Peptides and Amino Acids *13012
 Toxicology - General; Methods and Experimental *22501
 Physiology and Biochemistry of Bacteria *31000
 Genetics of Bacteria and Viruses 31500
 Medical and Clinical Microbiology - Bacteriology 36002
 BC Enterobacteriaceae 04810
 IT Miscellaneous Descriptors

MUTANT ANALYSIS A SUBUNIT INTERACTION

L10 ANSWER 15 OF 15 CAPLUS COPYRIGHT 2001 ACS
 AN 1994:597700 CAPLUS
 DN 121:197700

TI Assembly of *Escherichia coli* **heat-labile enterotoxin** and its secretion from *Vibrio cholerae*
 AU Sandkvist, Maria; Overbye, Linda J.; Sixma, Titia K.; Hol, Wim G.J.; Bagdasarian, Michael

CS Unit. Lab. Animal. Med., Univ. Michigan, Ann Arbor, MI, USA
 SO Dev. Plant Pathol. (1994), 3(Molecular Mechanisms of Bacterial Virulence),
 293-309

DT CODEN: DPPAEF
 Journal; General Review
 LA English
 CC 4-0 (Toxicology)
 AB A review with 64 refs. Subunits of the **heat-labile enterotoxin** of *Escherichia coli* (LT) assemble in the periplasm and

are secreted through the outer membrane in *Vibrio cholerae*. Deletions or substitutions of residues at the carboxyl terminus of the B subunit (**EtxB**) result in **mutant** polypeptides that assemble into normal pentamers at 30.degree.C but cannot assemble at 42.degree.C in vivo. This defect may be suppressed by substitutions of single amino

acid residues in regions that interact directly with the modified carboxyl terminus. Carboxyl terminal residues of **EtxB** thus appear to be required for formation or stabilization of an assembly intermediate of B subunit pentamerization but are not essential for the stability of the final pentamer. Secretion of the cholera toxin (CT) or of **EtxB** through the outer membrane of *V. cholerae* requires the functions of several genes that display extensive similarities to genes required for macromol. translocation in other Gram-neg. bacteria. One of the gene products required seems to be a cytoplasmic protein contg. ATP-binding domains. It may be a protein involved in the regulatory signal transduction.

ST review *Escherichia enterotoxin* assembly secretion *Vibrio*
 IT *Escherichia coli*
Vibrio cholerae

(assembly of *Escherichia coli* **heat-labile enterotoxin** and its secretion from *Vibrio cholerae*)
 IT Toxins
 RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
 (entero-, LT, assembly of *Escherichia coli* **heat-labile enterotoxin** and its secretion from *Vibrio cholerae*)

IT Biological transport
 (secretion, assembly of *Escherichia coli* **heat-labile enterotoxin** and its secretion from *Vibrio cholerae*)

=> d his

(FILE 'HOME' ENTERED AT 09:58:00 ON 02 MAR 2001)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 09:58:19 ON
02 MAR 2001

L1 36730 S ENTEROTOXIN
L2 5590 S L1 AND HEAT LABILE
L3 1 S L2 AND B SUBNIT
L4 649 S L2 AND MUTANT
L5 7 S L4 AND BINDING ASSAY
L6 3 DUP REMOVE L5 (4 DUPLICATES REMOVED)
L7 0 S L4 AND IL10
L8 0 S L4 AND REDUCE GM1 BINDING
L9 234 DUP REMOVE L4 (415 DUPLICATES REMOVED)
L10 15 S L9 AND ETXB
L11 1 S L10 AND CYTOKINE
L12 15 DUP REMOVE L10 (0 DUPLICATES REMOVED)

=> s 19 and TGF beta

L13 0 L9 AND TGF BETA

=> s 19 and mucosa immunity

L14 0 L9 AND MUCOSA IMMUNITY

=> s 19 and IL10

L15 0 L9 AND IL10

=> s 19 and cytokines

L16 11 L9 AND CYTOKINES

=> dup remove 116

PROCESSING COMPLETED FOR L16

L17 11 DUP REMOVE L16 (0 DUPLICATES REMOVED)

=> d all 117 1-11

L17 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2001 ACS

AN 2001:93811 CAPLUS

TI Potential mucosal adjuvants for human use

AU Ohmura, Mari; Jackson, Raymond J.; Takeda, Yoshifumi; McGhee, Jerry R.

CS The Immunobiology Vaccine Center, University Alabama, Birmingham, AL,
35294-2170, USA

SO Dev. Novel Antimicrob. Agents: Emerging Strategies (2001), 91-106.

Editor(s): Lohner, Karl. Publisher: Horizon Scientific Press, Wymondham,
UK.

CODEN: 69AXXR

DT Conference

LA English

CC 15 (Immunochemistry)

AB There have been no effective and safe adjuvants for use in humans since

aluminum compds. were approved by the US Federal Drug Administration. At present new adjuvants approved for use such as MDP and ISCOMs are limited to veterinary vaccines. Recent advances at the cellular and mol. levels of the immune system have led to the clin. application of certain **cytokines** for both immunotherapeutic and conventional vaccines.

While not yet in widespread use, the **cytokines** IL-2 and IL-12 hold potential promise for human adjuvant applications. A purified saponin, QS-21 is another promising adjuvant candidate that has proven safe in Phase I and Phase II human clin. trials. Finally, considerable effort has been focused on developing detoxified derivs. of bacterial **enterotoxins**. Thus, **mutants** of cholera toxin (CT)

produced by *Vibrio cholerae* and of the **heat labile enterotoxin** (labile toxin; LT) produced by enterotoxigenic *Escherichia coli*, which are non-toxic but which retain adjuvant activity have been constructed. Among these are CT E112K and S61F, which harbor single amino acid substitutions in the enzymically active A subunit and are promising and excellent adjuvant candidates for mucosal vaccination. These mols. are currently undergoing pre-clin. evaluation as potential mucosal adjuvants for use in humans. Thus, several potentially safe and effective mucosal adjuvants with the ability to redirect the immune

system

to a Th1 or Th2-type response are on the horizon.

RE.CNT 104

RE

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- (27) Eldridge, J; Semin Hematol Suppl 4
- (28) Eldridge, J; Semin Hematol P25
- (29) Eldridge, J; Semin Hematol 1993, V30(4), P16
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L17 ANSWER 2 OF 11 SCISEARCH COPYRIGHT 2001 ISI (R)

AN 2000:904194 SCISEARCH

GA The Genuine Article (R) Number: 376PZ

TI Interplay of **cytokines** and adjuvants in the regulation of mucosal and systemic HIV-specific CTL

AU Belyakov I M; Ahlers J D; Clements J D; Strober W; Berzofsky J A (Reprint)

CS NCI, MOL IMMUNOGENET & VACCINE RES SECT, METAB BRANCH, NIH, BLDG 10, ROOM 6B-12, MSC 1578, BETHESDA, MD 20892 (Reprint); NCI, MOL IMMUNOGENET & VACCINE RES SECT, METAB BRANCH, NIH, BETHESDA, MD 20892; TULANE UNIV, SCH MED, DEPT MICROBIOL & IMMUNOL, NEW ORLEANS, LA 70112; NIAID, MUCOSAL

IMMUN SECT, CLIN INVEST LAB, NIH, BETHESDA, MD 20892

CYA USA

SO JOURNAL OF IMMUNOLOGY, (1 DEC 2000) Vol. 165, No. 11, pp. 6454-6462. Publisher: AMER ASSOC IMMUNOLOGISTS, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.

ISSN: 0022-1767.

DT Article; Journal

FS LIFE

LA English

REC Reference Count: 71

AB We examined the interplay between **cytokines** and adjuvants to optimize the induction of CTL by a mucosal HIV peptide vaccine. We show synergy between IL-12 and GM-CSF when administered together with the HIV peptide PCLUS3-18IIIB and cholera toxin (CT) in the induction of CTL activity and protection against mucosal viral transmission. Further, we examine the efficacy of **mutant** Escherichia coli labile toxin, LT(R192G), as a less toxic adjuvant than CT. LT(R192G) was as effective

as or more effective than CT at inducing a mucosal CTL response. Moreover, LT(R192G) was as effective without IL-12 as CT was when combined with IL-12, and the response elicited by LT(R192G) with the vaccine was not further enhanced by the addition of IL-12. GM-CSF synergized, with LT(R192G) without exogenous IL-12. Therefore, LT(R192G) may induce a more favorable cytokine response by not inhibiting IL-12 production. In particular, less IL-4 is made after LT(R192G) than CT immunization, and the response is less susceptible to anti-IL-12 inhibition. Thus, the choice of mucosal adjuvant affects the cytokine environment, and the mucosal response and protection can be enhanced by manipulating the cytokine environment with synergistic cytokine combinations incorporated in the vaccine.

CC IMMUNOLOGY

STP KeyWords Plus (R): **HEAT-LABILE ENTEROTOXIN;**
 ADP-RIBOSYLTRANSFERASE ACTIVITY; CYTOTOXIC T-LYMPHOCYTES;
 HUMAN-IMMUNODEFICIENCY-VIRUS; ESCHERICHIA-COLI **ENTEROTOXIN;**
 CHOLERA-TOXIN; CLASS-I; A-SUBUNIT; NONTOXIC **MUTANT;** TH2 CELLS

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TAKAHASHI H	1988	85	3105	P NATL ACAD SCI USA
TAKAHASHI I	1996	173	627	J INFECT DIS
TAKESHITA T	1995	154	1973	J IMMUNOL
TRIBBLE D R	1997			37 INT C ANT AG CHEM
TRINCHIERI G	1998	16	365	INT REV IMMUNOL
TSUJI T	1991	291	319	FEBS LETT
TSUJI T	1990	265	22520	J BIOL CHEM
XUAMANO J C	1993	178	1309	J EXP MED
YAMAMOTO S	1997	185	1203	J EXP MED
YAMAMOTO S	1997	94	5267	P NATL ACAD SCI USA

L17 ANSWER 3 OF 11 BIOSIS COPYRIGHT 2001 BIOSIS

AN 2001:13340 BIOSIS

DN PREV200100013340

TI Intranasal immunization of mice with CpG DNA induces strong systemic and mucosal responses that are influenced by other mucosal adjuvants and antigen distribution.

AU McCluskie, Michael J.; Weeratna, Risini D.; Davis, Heather L. (1)

CS (1) Loeb Health Research Institute, Ottawa Hospital, 725 Parkdale Avenue, Ottawa, K1Y 4E9: hdavis@LRI.ca Canada

SO Molecular Medicine (New York), (October, 2000) Vol. 6, No. 10, pp. 867-877. print.
ISSN: 1076-1551.

DT Article

LA English

SL English

AB Background: Synthetic oligodeoxynucleotides (ODN) containing immunostimulatory cytosine-guanine phosphate-linked dinucleotide (CpG) motifs are potent systemic and mucosal adjuvants in mice that have synergistic action with numerous other adjuvants, including alum and cholera toxin (CT). Herein, we evaluate CpG ODN with intranasal (IN) delivery of purified hepatitis B surface antigen (HBsAg), relative to and in combination with CT, Escherichia coli **heat labile enterotoxin** (LT), the B subunit of CT (CTB), and a nontoxic derivative of LT (LTK63). Materials and Methods: BALB/c mice were immunized by IN administration of HBsAg, alone or combined with CT, LT, CTB, or LTK63, and/or CpG ODN, or non-CpG control ODN. In addition, the effect of low-or high-volume administration was assessed, in order to target upper respiratory or entire respiratory tract, respectively. HBsAg-specific systemic (immunoglobulins: IgG, IgG1, IgG2a in plasma) and mucosal (IgA in fecal, lung, vaginal, saliva, and gut samples) humoral responses, as well as cell-mediated immune responses including T-cell proliferation and **cytokines** (interleukins: IL-4, IL-5; interferon: IFN-gamma) were evaluated. Results: CpG ODN, CT, and LT augmented anti-HBs titers equally, and more so than did CTB or LTK63. CpG ODN acted synergistically with CT and LT, but not CTB or LTK63 to enhance anti-HBs titers. Nevertheless, CpG ODN induced a more Th1-like response

for all combinations, compared with the same formulation without CpG. Strength of induced systemic and mucosal immune responses was better with IN delivery of a large volume. A small volume required multiple administrations and higher doses of antigen and adjuvant for equal results. This suggests that delivery of antigen to the lung and/or digestive system is superior to delivery to the nasal cavity. Conclusions: Our results suggest that the synergy between CpG ODN and native toxins (CT, LT) may depend on their enzymatic activity and that

- the lack of synergy with nontoxic derivatives (LTB, LTK63) arises, since they do not have enzymatic activity. Because both CT and LT are too toxic for use in humans, it is possible that CpG ODN may be combined with bacterial toxin **mutants** that retain some enzymatic activity to optimize immune augmentation.
- CC Immunology and Immunochemistry - General; Methods *34502
Cytology and Cytochemistry - Animal *02506
Biochemical Studies - General *10060
Biochemical Studies - Proteins, Peptides and Amino Acids *10064
Blood, Blood-Forming Organs and Body Fluids - Blood and Lymph Studies *15002
Blood, Blood-Forming Organs and Body Fluids - Blood Cell Studies *15004
Respiratory System - Physiology and Biochemistry *16004
- IT Major Concepts
Biochemistry and Molecular Biophysics; Immune System (Chemical Coordination and Homeostasis)
- IT Parts, Structures, & Systems of Organisms
T cell: blood and lymphatics, immune system, proliferation; nasal cavity: respiratory system; upper respiratory tract: respiratory system
- IT Chemicals & Biochemicals
CpG; Escherichia coli **heat labile enterotoxin**; alum; anti_HBs titer; antigen: distribution; cholera toxin; **cytokines**; hepatitis B surface antigen; native toxins; synthetic oligodeoxynucleotides: immunostimulatory cytosine-guanine phosphate-linked dinucleotide motif
- IT Methods & Equipment
CT [computed tomography]: imaging method
- IT Miscellaneous Descriptors
immune augmentation; mucosal immune response
- ORGN Super Taxa
Muridae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia
- ORGN Organism Name
BALB/c mouse (Muridae)
- ORGN Organism Superterms
Animals; Chordates; Mammals; Nonhuman Mammals; Nonhuman Vertebrates; Rodents; Vertebrates
- RN 10043-01-3Q (ALUM)
10043-67-1Q (ALUM)
- L17 ANSWER 4 OF 11 MEDLINE
AN 1999386826 MEDLINE
DN 99386826
TI Genetically detoxified **mutants** of **heat-labile** toxin from Escherichia coli are able to act as oral adjuvants.
- AU Douce G; Giannelli V; Pizza M; Lewis D; Everest P; Rappuoli R; Dougan G
CS Department of Biochemistry, Imperial College of Science, Technology and Medicine, London SW7 2AY, United Kingdom.
SO INFECTION AND IMMUNITY, (1999 Sep) 67 (9) 4400-6.

Journal code: G07. ISSN: 0019-9567.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

EM 199912

EW 19991202

AB Detoxified **mutants** of the Escherichia coli **heat-labile** toxin (LT) act as mucosal adjuvants to intranasally presented coadministered antigens. Here, we compare the adjuvant activity of a panel of detoxified derivatives of LT, using both intranasal (i.n.) and oral (p.o.) routes of administration. The **mutants** used as adjuvants varied in sensitivity to proteases and toxicity. With keyhole limpet hemocyanin (KLH) as the bystander antigen, the immune responses to i. n. immunizations were consistently higher than the equivalent p.o. -delivered proteins. LT-G192, a **mutant** which demonstrates a 10-fold reduction in toxicity in vitro, demonstrated wild-type adjuvant activity both i.n. and p.o., inducing similar titers of KLH specific antibody in the sera and immunoglobulin A in local mucosal secretions as wild-type LT. In line with previous data, the nontoxic holotoxoid LT-K63 induced intermediate immune responses in both the serum and mucosal secretions which were lower than those achieved with wild-type LT but at least 10-fold higher than those measured when the antigen was administered with LT-B. Although significant levels of local and systemic anti-KLH antibodies were induced following p.o. immunization with LT-K63, cellular proliferative responses to KLH was poor or undetectable. In contrast, LT and LT-G192 induced significant T-cell responses to KLH following p.o. immunization. These proliferating cells secreted both gamma interferon and interleukin-5, suggesting that the type of immune response induced following p.o. coimmunization with LT and purified protein is a mixed Th1/Th2 response.

CT Check Tags: Animal; Female; Support, Non-U.S. Gov't

*Adjuvants, Immunologic
Administration, Intranasal
Administration, Oral
Bacterial Toxins: AD, administration & dosage
Bacterial Toxins: GE, genetics
*Bacterial Toxins: IM, immunology
Bacterial Toxins: ME, metabolism
Cell Division
Cells, Cultured
Cytokines: BI, biosynthesis
Enterotoxins: AD, administration & dosage
Enterotoxins: GE, genetics
*Enterotoxins: IM, immunology
Enterotoxins: ME, metabolism
*Escherichia coli
Escherichia coli: GE, genetics
Hemocyanin: AD, administration & dosage
*Hemocyanin: IM, immunology
Immunoglobulin Isotypes
Mice
Mice, Inbred BALB C
Mutagenesis, Site-Directed
Spleen: CY, cytology
Trypsin: ME, metabolism

RN 9013-72-3 (Hemocyanin)
 CN EC 3.4.21.4 (Trypsin); 0 (**enterotoxin** LT); 0 (keyhole-limpet hemocyanin); 0 (Adjuvants, Immunologic); 0 (Bacterial Toxins); 0 (**Cytokines**); 0 (**Enterotoxins**); 0 (Immunoglobulin Isotypes)

L17 ANSWER 5 OF 11 MEDLINE
 AN 1999429341 MEDLINE
 DN 99429341
 TI The role of cAMP in mucosal adjuvanticity of Escherichia coli **heat-labile enterotoxin** (LT).
 AU Cheng E; Cardenas-Freytag L; Clements J D
 CS Department of Microbiology and Immunology, Tulane University Medical Center, New Orleans, LA 70112-2699, USA.
 NC AI42777 (NIAID)
 SO VACCINE, (1999 Aug 20) 18 (1-2) 38-49.
 Journal code: X60. ISSN: 0264-410X.
 CY ENGLAND: United Kingdom
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 200001
 EW 20000104
 AB **Heat-labile enterotoxin** (LT) produced by enterotoxigenic Escherichia coli (ETEC) and cholera toxin (CT) produced by Vibrio cholerae have been shown to function as potent mucosal adjuvants.

A number of studies have examined the effects of different mutations at either the active site or the protease site of LT and CT and the influence of those mutations on toxicity and adjuvanticity. However, different observations reported by various groups using a variety of animal models with different antigens or different routes of immunization have provided contradictory findings and evoked many questions regarding the underlying mechanisms of mucosal adjuvanticity of LT and CT. In this study, the role of cAMP in mucosal adjuvanticity was examined by comparing three LT active site mutants (S61F, A69G, E112K), a protease site mutant (R192G) and recombinant LT-B for toxicity, cAMP activity and mucosal adjuvanticity using tetanus toxoid (TT) as a model antigen. While all mutants examined showed reduced toxicity, the effects of each mutation on its ability to function as an adjuvant varied. Following intranasal immunization, native LT as well as protease and active site mutants of LT induced serum anti-TT IgG and their responses were virtually indistinguishable from one another. In addition, LT-B was also able to enhance production of serum anti-TT IgG, though at a level significantly lower than that achieved by native LT and mutants. Following oral immunization, the best serum anti-TT IgG responses were obtained with native LT and mutants that retained the ability to induce accumulation of cAMP. Despite the nearly identical serum anti-TT IgG responses following intranasal immunization, there was a strong correlation between the ability to induce accumulation of cAMP in cultured Caco-2 cells and the ability to elicit production of antigen-specific Th1 or Th2 **cytokines**.

CT Check Tags: Animal; Female; Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

*Adjuvants, Immunologic: PD, pharmacology
Administration, Oral

*Bacterial Toxins: PD, pharmacology
Caco-2 Cells

*Cyclic AMP: PH, physiology

Cytokines: BI, biosynthesis

*Enterotoxins: PD, pharmacology

*Escherichia coli: PY, pathogenicity

IgG: BL, blood

Immunization

Mice

Mice, Inbred BALB C

Mutagenesis, Site-Directed

Structure-Activity Relationship

Tetanus Toxoid: IM, immunology

RN 60-92-4 (Cyclic AMP)

CN 0 (**enterotoxin** LT); 0 (Adjuvants, Immunologic); 0 (Bacterial
Toxins); 0 (**Cytokines**); 0 (**Enterotoxins**); 0 (IgG); 0
(Tetanus Toxoid)

L17 ANSWER 6 OF 11 MEDLINE

AN 1998187895 MEDLINE

DN 98187895

TI Glycosphingolipids as novel targets for T-cell suppression by the B
subunit of recombinant **heat-labile enterotoxin**

AU Truitt R L; Hanke C; Radke J; Mueller R; Barbieri J T
CS Department of Pediatrics, Cancer Center, Medical College of Wisconsin,
Milwaukee 53226, USA.. rtruitt@mcw.edu

NC CA73738 (NCI)

AI30162 (NIAID)

SO INFECTION AND IMMUNITY, (1998 Apr) 66 (4) 1299-308.
Journal code: GO7. ISSN: 0019-9567.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

EM 199806

EW 19980602

AB **Heat-labile enterotoxin** subunit B (LTB) is a
noncatalytic protein derived from Escherichia coli that binds to
ganglioside GM1, a glycosphingolipid on the surface of mammalian cells.

In this study, the effects of recombinant LTB (rLTB) on murine lymphocytes
were examined in vitro. T and B cells readily bound fluorescein
isothiocyanate-labeled rLTB. CD8+ T cells bound twice as much as CD4+ T
cells and B cells. Exposure of T-cell subsets and B cells to rLTB
abrogated mitogen-driven proliferation. CD8+ T cells were more
susceptible

to rLTB than either CD4+ T cells or B cells. There were differences in

the sensitivity of lymphocytes from various strains of mice to rLTB. This was
attributed to qualitative and quantitative differences in the CD4+ T
cells. rLTB induced apoptosis in both T-cell subsets, but the level was
significantly higher in CD8+ T cells. Apoptosis peaked at around 8 h

after exposure to rLTB and incubation at 37 degrees C. Binding to ganglioside
GM1 was essential for suppression, since rLTB/G33D, a **mutant**

which does not bind GM1, failed to inhibit proliferation or induce apoptosis. Naive T cells, which were acutely sensitive to rLTB, became more resistant after activation. Conversely, activated T cells regained their sensitivity to rLTB when they reverted back to a resting state. A 1-h pulse with rLTB was sufficient to inhibit T-cell proliferation and cytotoxic-T-lymphocyte generation in primary mixed lymphocyte reaction cultures. CD8+ T cells were preferentially depleted in these cultures. rLTB also induced functional modifications in T cells as indicated by inhibition of gamma interferon secretion after polyclonal activation. Thus, rLTB may have immunomodulatory properties independent of its

ability

to induce apoptosis.

CT Check Tags: Animal; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Apoptosis: DE, drug effects

*Bacterial Toxins: PD, pharmacology

Cytokines: BI, biosynthesis

*Enterotoxins: PD, pharmacology

*G(M1) Ganglioside: PH, physiology

*Immunosuppressive Agents: PD, pharmacology

Lymphocyte Culture Test, Mixed

Lymphocyte Transformation: DE, drug effects

Mice

Mice, Inbred AKR

Mice, Inbred BALB C

Mice, Inbred C57BL

Mice, Inbred DBA

Recombinant Proteins: PD, pharmacology

*T-Lymphocytes: DE, drug effects

RN 37758-47-7 (G(M1) Ganglioside)

CN 0 (**enterotoxin** LT); 0 (Bacterial Toxins); 0 (**Cytokines**); 0 (**Enterotoxins**); 0 (Immunosuppressive Agents); 0 (Recombinant Proteins)

L17 ANSWER 7 OF 11 MEDLINE

AN 1998223787 MEDLINE

DN 98223787

TI LT(R192G), a non-toxic **mutant** of the **heat-labile enterotoxin** of Escherichia coli, elicits enhanced humoral and cellular immune responses associated with protection against lethal oral challenge with Salmonella spp.

AU Chong C; Friberg M; Clements J D

CS Department of Microbiology and Immunology, Tulane University Medical Center, New Orleans, LA 70112, USA.

NC AI28835 (NIAID)

AI36519 (NIAID)

SO VACCINE, (1998 Apr) 16 (7) 732-40.
Journal code: X60. ISSN: 0264-410X.

CY ENGLAND: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199808

AB In the current study we examined the ability of a novel mucosal adjuvant, LT(R192G), to enhance the humoral and cellular immune responses against killed Salmonella spp. and to affect protection against lethal oral challenge with wild-type organisms. Mice orally immunized with killed S. dublin in conjunction with LT(R192G) were protected against lethal oral challenge and had higher IFN-gamma, IL-2 and IgG responses than did mice

orally immunized with killed S. dublin alone which were not protected. This study demonstrates that the function of LT(R192G) in protection against typhoid-like disease is to upregulate/enhance the Th1 arm of the immune response against killed organisms. When used as a mucosal

adjuvant,

LT(R192G) enables the use of killed bacteria or viruses as vaccines by enhancing the overall humoral and cellular host immune response to these organisms, especially the Th1 arm of the immune response. These findings have significant implications for vaccine development and further support the potential of LT(R192G) to function as a safe, effective adjuvant for mucosally administered vaccines.

CT Check Tags: Animal; Female; Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.

Administration, Oral

Antibodies, Bacterial: BI, biosynthesis

Antibodies, Bacterial: BL, blood

Antibody Formation: DE, drug effects

Antibody Formation: IM, immunology

Antibody Specificity

Bacterial Toxins: GE, genetics

Bacterial Toxins: IM, immunology

*Bacterial Toxins: TU, therapeutic use

Cytokines: BI, biosynthesis

Enterotoxins: GE, genetics

Enterotoxins: IM, immunology

***Enterotoxins: TU, therapeutic use**

Feces

Immunity, Cellular: DE, drug effects

Immunity, Cellular: IM, immunology

Immunity, Mucosal: DE, drug effects

Immunity, Mucosal: IM, immunology

Interferon Type II: IM, immunology

Leukocytes, Mononuclear: ME, metabolism

Lipopolysaccharides: IM, immunology

Lymph Nodes: CY, cytology

Lymph Nodes: IM, immunology

Mice

Mice, Inbred BALB C

Mutation

Neutralization Tests

*Salmonella Infections, Animal: IM, immunology

*Salmonella Infections, Animal: PC, prevention & control

Spleen: CY, cytology

Spleen: IM, immunology

RN 82115-62-6 (Interferon Type II)

CN 0 (**enterotoxin** LT); 0 (Antibodies, Bacterial); 0 (Bacterial Toxins); 0 (**Cytokines**); 0 (**Enterotoxins**); 0 (Lipopolysaccharides)

L17 ANSWER 8 OF 11 SCISEARCH COPYRIGHT 2001 ISI (R)

AN 97:312966 SCISEARCH

GA The Genuine Article (R) Number: WU183

TI **Mutants** in the ADP-ribosyltransferase cleft of cholera toxin lack diarrheagenicity but retain adjuvant activity

AU Yamamoto S; Takeda Y; Yamamoto M; Kurazono H; Imaoka K; Yamamoto M; Fujihashi K; Noda M; Kiyono H; McGhee J R (Reprint)

CS UNIV ALABAMA, IMMUNOBIOLOGICAL VACCINE CTR, 845 19TH ST S, BIRMINGHAM, AL 35294 (Reprint); UNIV ALABAMA, IMMUNOBIOLOGICAL VACCINE CTR, BIRMINGHAM, AL 35294;

UNIV ALABAMA, DEPT MICROBIOL, BIRMINGHAM, AL 35294; UNIV ALABAMA, DEPT ORAL BIOL, BIRMINGHAM, AL 35294; KYOTO UNIV, FAC MED, DEPT MICROBIOL, KYOTO 606, JAPAN; INT MED CTR JAPAN, RES INST, SHINJUKU KU, TOKYO 162, JAPAN; CHIBA UNIV, FAC MED, DEPT MICROBIOL 2, CHUO KU, CHIBA 260, JAPAN; OSAKA UNIV, MICROBIAL DIS RES INST, DEPT MUCOSAL IMMUNOL, SUITA, OSAKA 565, JAPAN

CYA USA; JAPAN

SO JOURNAL OF EXPERIMENTAL MEDICINE, (7 APR 1997) Vol. 185, No. 7, pp. 1203-1210.
 Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021.
 ISSN: 0022-1007.

DT Article; Journal

FS LIFE

LA English

REC Reference Count: 38

AB Cholera toxin (CT), the most commonly used mucosal adjuvant in experimental animals, is unsuitable for humans because of potent diarrhea-inducing properties. We have constructed two CT-A subunit mutants, e.g., serine-->phenylalanine at position 61 (S61F), and glutamic acid-->lysine at 112 (E112K) by site-directed mutagenesis. Neither mutant CT (mCT), in contrast to native CT (nCT), induced adenosine diphosphate-ribosylation, cyclic adenosine monophosphate formation, or fluid accumulation in ligated mouse ileal loops. Both mCTs retained adjuvant properties, since mice given ovalbumin (OVA) subcutaneously with mCTs or nCT, but not OVA alone developed high-titered serum anti-OVA immunoglobulin G (IgG) antibodies (Abs) which were largely of IgG1 and IgG2b subclasses. Although nCT induced brisk IgE Ab responses, both mCTs elicited lower anti-OVA IgE Abs. OVA-specific CD4(+) T cells were induced by nCT and by mCTs, and quantitative analysis of secreted cytokines and mRNA revealed a T helper cell 2 (Th2)-type response. These results now show that the toxic properties of CT can be separated from adjuvanticity, and the mCTs induce Ab responses via a Th2 cell pathway.

CC IMMUNOLOGY; MEDICINE, RESEARCH & EXPERIMENTAL

STP KeyWords Plus (R): **HEAT-LABILE ENTEROTOXIN;**
 ESCHERICHIA-COLI; B-SUBUNIT; A-SUBUNIT; GANGLIOSIDE GM1; VIBRIO-CHOLERA; TH2 CELLS; MICE; PROTEIN; VACCINE

RF 95-4226 004; MUCOSAL IMMUNIZATION; DIPHTHERIA-TOXIN RECEPTOR; INDUCTION

OF A SECRETORY IGA RESPONSE; FEMALE MICE; PULMONARY IMMUNITY; ORAL COLONIZATION

RE

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DOUCE G	1995	92	1644	P NATL ACAD SCI USA
ELSON C O	1984	132	2736	J IMMUNOL
FIELD M	1989	321	800	NEW ENGL J MED
FUJITA K	1972	125	647	J INFECT DIS
FUKUTA S	1988	56	1748	INFECT IMMUN
GILL D M	1976	15	1242	BIOCHEMISTRY-US
GILL D M	1981	33	677	INFECT IMMUN

GILL D M	1975	250	6424	J BIOL CHEM
GUERRANT R L	1974	10	320	INFECT IMMUN
HARFORD S	1989	183	311	EUR J BIOCHEM
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KUNKEL T A	1987	154	367	METHOD ENZYMOL
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LEE H J	1993	151	6135	J IMMUNOL
LYCKE N	1992	22	2277	EUR J IMMUNOL
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MARINARO M	1995	155	4621	J IMMUNOL
MCGEE D W	1993	61	4637	INFECT IMMUN
MEKALANOS J J	1983	306	551	NATURE
MUNOZ E	1990	172	95	J EXP MED
NODA M	1989	28	7936	BIOCHEMISTRY-US
SAKAGUCHI M	1989	190	189	J IMMUNOL METHODS
SIXMA T K	1991	351	371	NATURE
SPANGLER B D	1992	56	622	MICROBIOL REV
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TAMURA S	1988	6	409	VACCINE
TSUJI T	1990	265	22520	J BIOL CHEM
VAJDY M	1995	181	41	J EXP MED
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XUAMANO J C	1993	178	1309	J EXP MED
YAMAMOTO M	1996	148	331	AM J PATHOL

L17 ANSWER 9 OF 11 MEDLINE

AN 96217741 MEDLINE

DN 96217741

TI Oral immunization with the B subunit of the **heat-labile enterotoxin** of Escherichia coli induces early Th1 and late Th2 cytokine expression in Peyer's patches.

AU Nakagawa I; Takahashi I; Kiyono H; McGhee J R; Hamada S

CS Department of Oral Microbiology, Osaka University, Faculty of Dentistry, Japan.

NC AI-15128 (NIAID)

AI-18958 (NIAID)

SO JOURNAL OF INFECTIOUS DISEASES, (1996 Jun) 173 (6) 1428-36.
Journal code: IH3. ISSN: 0022-1899.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Abridged Index Medicus Journals; Priority Journals

EM 199609

AB The B subunit of **heat-labile** toxin (LT-B) from enterotoxigenic Escherichia coli has been shown to be a powerful mucosal immunogen. Oral immunization of mice with LT-B revealed that BALB/c

(H-2d)

and C57BL/6 (H-2b) mice gave high serum IgG and mucosal IgA responses specific for LT-B. However, ALY (H-2b) mice lacking intestinal Peyer's patches (PP) did not respond to oral LT-B with either serum or mucosal antibodies. These results indicate that PP lymphocytes supported both systemic and mucosal immune responses when the antigen was administered orally. Reverse transcription polymerase chain reaction analyses revealed that PP CD4 T cells expressed early Th1-type (interferon-gamma and interleukin [IL]-2) and late Th2-type (IL-4, -5, and -6) cytokine mRNA.